

The Effects of Anterior Cruciate Ligament and Synovial Tissue on Responses of Patellar and Quadriceps Tendon to Inflammatory Stimulation

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Disclosures: Kelsey R. Atteberry (N), Luke Annin (N), Maria L. Hinkamp (N), Chance Strickler (N), Hailee C. Coleman (N), Gillian M. Hark (N), James L. Cook (1-Arthrex, MTF; 2-Arthrex; 3B-Arthrex, Bioventus, Collagen Matrix Inc, Trupanion.; 5-Arthrex, Collagen Matrix Inc, Celularity, MTF, NIH, Organogenesis, U.S. DOD, Zimmer-Biomet; 7B-Thieme; 8-J of Knee Surgery; 9-MTN, MTF), Aaron M. Stoker (1-MTF)

Introduction: Rupture of the anterior cruciate ligament (ACL) is a common injury that is most commonly treated by ACL reconstruction (ACLR) using tendon autografts or allografts with patellar tendon (PT) or quadriceps tendon (QT) autografts being among the most frequently used. While ACLR is considered generally successful, failures still occur in 10%-15% of patients. Among the factors that can contribute to ACLR failure, tendon autograft metabolic responses to the pro-inflammatory and pro-degradative environment in the injured joint during the process of "ligamentization" may be key determinants of ACLR success or failure, but have not been fully characterized to date. Therefore, this study was designed to evaluate the effects of key contributors to the intra-articular environment, ACL and synovium, on metabolic responses of PT and QT tissues to inflammatory stimulation. It was hypothesized that PT and QT tendons will have significantly higher pro-inflammatory and pro-degradative responses to IL-1 β stimulation compared to untreated mono-culture and co-cultured controls, and that PT and QT tissues co-cultured with synovium or ACL will be associated with significantly higher pro-inflammatory and pro-degradative responses compared to mono-cultured tissues with or without IL-1 β stimulation.

Methods: With ACUC approval, anterior cruciate ligament (ACL), synovial (SYN), quadriceps tendon (QT) and patellar tendon (PT) tissues were recovered from purpose bred research hounds (n=5) euthanized for reasons unrelated to this study. Tissue explants (6mm) were created for each tissue type and cultured for 24 hours. After 24 hours, PT and QT explants were cultured alone (MONO) or co-cultured with ACL (COA) or SYN (COS) tissue explants with or without IL-1 β (1ng/ml) stimulation for 6 days. Culture media were changed and collected for biomarker analysis every 3 days of culture. On day 6 of culture, tissues were flash frozen in LN₂ and stored at -80°C. Total mRNA was extracted from the tissues using the Qiagen RNeasy Plus Mini Kit. Gene expression levels relative to GAPDH were determined for COL I, COL III, Decorin, Elastin, COMP, Aggrecan, SCX, MMP-1, MMP-2, MMP-3, ADAMTS4, TIMP-1, TIMP-2, TIMP-3, and COX-2 using the QuantiNova SYBR green real time RT-PCR kit. Culture media were tested for concentrations of MMP-1, MMP-2, MMP-3, MMP-13, KC, Gro- α , IL-6, IL-8, NO, and PGE2 using commercially available assays. Significant differences (p<0.05) between PT or QT tissues treated with and without IL-1 β stimulation in each culture group, between PT or QT tissues in the mono-culture group and each co-culture group, and between QT and PT tissues in each culture group were determined using a Mann-Whitney rank sum Test.

Results: Responses to IL-1 β : In response to IL-1 β stimulation, the PT and QT explants were associated with significantly higher relative expression of COX-2 and MMP-1, significantly lower relative expression of AGG, and significantly higher production of PGE2, IL-6, IL-8, NO, MMP-3 and MMP-13 on days 3 and/or 6 of culture in all groups. (Fig. 1) The relative expression of COL I was significantly lower in the PT MONO and PT and QT COA groups. **Effects of Co-culture (Fig. 2):** PT explants cultured in the COS group had significantly lower relative expression of COL I, MMP-2, MMP-3, and ADAMTS4, without IL-1 β treatment, and significantly higher relative expression COMP with IL-1 β treatment, compared to the MONO group. PT explants cultured in the COA group had significantly lower relative expression levels of COL I without IL-1 β treatment and significantly higher relative expression levels of COMP with or without IL-1 β treatment compared to the MONO group. QT explants cultured in the COS group had significantly lower relative expression of COL I with and without IL-1 β treatment compared to the MONO group. QT explants cultured in the COA group had significantly lower relative expression of COL I without IL-1 β , and significantly higher relative expression of ADAMTS4, compared to the MONO group. **Patellar vs. Quadriceps Tendon Explants:** PT explants had significantly higher relative expression of COL I, and significantly lower relative expression of COX-2, and produced lower levels of IL-8 and KC in the MONO group, compared to the QT without IL-1 β treatment. In the MONO group, PT explants had significantly higher relative expression of COL I compared to the QT without IL-1 β , and significantly higher relative expression of MMP-2 and MMP-3 with IL-1 β , compared to QT explants. However, the QT produced higher levels of MMP-1 and MMP-3 with or without IL-1 β , compared to PT tissues. In the COS group, PT explants had significantly higher relative expression of COMP, SCX, and DEC with IL-1 β , and produced significantly higher levels of MMP-2 without IL-1 β on day 6, compared to the QT explants. In the COA group, QT tissues produced significantly higher levels of MMP-3 without IL-1 β compared to PT tissues.

Discussion: The data from this study indicated that IL-1 β stimulation significantly influences pro-inflammatory and pro-degradative responses of PT and QT explants during *in vitro* culture. COL I gene expression in PT and QT explants was significantly lower in co-cultures with ACL and synovial tissues, indicating that the intra-articular environment may have significant negative effects on matrix production by tendon autografts after ACLR. However, ACL and synovial tissues had minimal effects on the pro-inflammatory and pro-degradative responses of the PT and QT explants in this model, suggesting that inflammatory and degradative pathways may not significantly impact metabolic responses of tendon autografts after ACLR. Interestingly, quadriceps tendon autografts may have higher basal pro-inflammatory mediator production than patellar tendons based on the findings of higher COX-2 expression and production of IL-8 and KC in QT explants noted in this study. Additionally, PT explants were associated with significantly higher COL I and MMP-2 relative expression levels and significantly lower levels of MMP-1 during culture when compared to QT explants, suggesting inherent differences in matrix production and turnover between these two tendons. While MMP-3 gene expression was significantly higher in PT explants, the production of MMP-3 during culture was significantly higher in QT explants, indicating potential MMP-3 regulatory and/or translation differences between PT and QT. Ongoing studies in our lab are aimed at further characterization of these differences through *in vitro*, preclinical and clinical studies in order to optimize graft choice for ACLR surgeries.

Significance: The data from this study provide insight into the responses of patellar tendon and quadriceps tendon autografts in the intra-articular environment following ACLR. Further characterization of these factors that can influence the process of "ligamentization" may improve graft selection and outcomes for patients undergoing ACLR surgery.